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Title: IDENTIFICATION OF ACTINOBACILLUS ACTINOMYCETEMCOMITANS ANTIGENS FOR USE IN THE DIAGNOSIS, TREATMENT, AND MONITORING OF PERIODONTAL DISEASES

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IDENTIFICATION OF ACTINOBACILLUS ACTINOMYCETEMCOMITANS ANTIGENS FOR USE IN THE DIAGNOSIS, TREATMENT, AND MONITORING OF PERIODONTAL DISEASES

GOVERNMENT INTERESTS

This invention was made with Government support under Grant Number RO1 DE13523 awarded by the National Institutes of Health (National Institute for Dental and Craniofacial Research, NIDCR). The Government has certain rights in the invention.

TECHNICAL AREA OF THE INVENTION

This invention provides methods and compositions for the diagnosis, treatment, preventions, and amelioration of diseases caused by *Actinobacillus actinomycetemcomitans*.

BACKGROUND OF THE INVENTION

Actinobacillus actinomycetemcomitans (Aa) is the principal etiologic agent of early-onset periodontitis including localized and generalized prepubertal periodontis, localized and generalized juvenile periodontis, and rapidly progressive or refractory adult periodontitis. Currently, diagnosis of these diseases is made by X-ray analysis usually long after the onset of the disease and after considerable damage to the supporting bone and tissue has occurred. Tooth loss is the ultimate detrimental effect of destructive periodontal disease. A national survey of the United States revealed a prevalence of localized juvenile periodontitis of 0.53% and of generalized juvenile periodontitis of 0.13%. Loe & Brown, *J. Periodontol.* 62:608-616 (1991). Findings from a number of studies corroborate the conclusion that early-onset disease is similar in other industrialized countries and is more frequent in developing countries. Loe & Brown, *J. Periodontol.* 62:608-616 (1991). Therefore, methods of early diagnosis of early-onset

periodontitis, localized and generalized juvenile periodontis, and rapidly progressive or refractory adult periodontitis are needed in the art. In addition, certain types of adult periodontitis, which in general is a very common condition affecting over half the adult population, are likely to be caused by Aa. Furthermore, Aa can cause extra-oral diseases such as endocarditis, thyroid gland abscesses, urinary tract infections, brain abscesses, and vertebral osteomyelitis.

There are antibiotic, surgical, and mechanical therapies for the treatment of Aa induced periodontis, but no means for prevention. Tetracycline has been widely used in the treatment of early-onset periodontis. There remains a concern, however, of strains developing resistance to tetracycline as well as the possibility of overgrowth of other pathogenic microorganisms. Given the incidence of these diseases, a safe vaccine for Aa is needed. A vaccine can be, for example, a multivalent vaccine. Control of periodontal disease is also very important in light of recent attention to the possible role of periodontal infections as risk factors for systemic disease (e.g., coronary heart disease).

While most people have Aa as a normal member of their dental plaque, it usually does not cause disease. However, when Aa does cause disease, the host mounts an enormous immune response that is inevitably futile, presumably because the immune response is directed against the wrong Aa antigens. Providing the most appropriate periodontal treatment requires making an accurate diagnosis, performing optimum treatment, and monitoring the patient's response to therapy.

Currently, standard microbiological tests for Aa detect only the presence of Aa in dental plaque, and do not specifically identify disease activity. For this reason these tests have a low positive predictive value. Because Aa is normally found in the plaque of even healthy

individuals, the application of these tests is limited in their usefulness to those who present with certain clinical manifestations of disease, including for example, patients with advanced attachment loss and bone loss before the age of 25, patients aged about 25-35 with rapid destruction of attachment and bone in a relatively short period of time (rapidly progressive periodontitis), and patients who continue to lose attachment despite stringent treatment (refractory periodontitis).

DNA probe technology has been developed to identify the presence of Aa in dental plaque, but this technology is unable to distinguish between Aa that is normally part of the dental plaque community and Aa that is involved in an actual disease process. Therefore, these DNA probes do not identify Aa involved in a disease process.

Therefore, methods of diagnosing, monitoring, treating, preventing, or ameliorating a disease caused by Aa are needed in the art.

SUMMARY OF THE INVENTION

It is an object of the invention to provide methods and compositions for the treatment, amelioration, and prevention of diseases caused by Aa. This and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention provides a purified immunogenic polypeptide comprising at least 5 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46,

SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:140, SEQ ID NO:142, SEQ ID NO:144, SEQ ID NO:146, SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:152, SEQ ID NO:154, SEQ ID NO:156, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:162, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:170, SEQ ID NO:172, SEQ ID NO:174, SEQ ID NO:176, SEQ ID NO:178, SEQ ID NO:180, SEQ ID NO:182, SEQ ID NO:184, SEQ ID NO:186, SEQ ID NO:188, SEQ ID NO:190, SEQ ID NO:192, SEQ ID NO:194, SEQ ID NO:196, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:208, SEQ ID NO:210, SEQ ID NO:212, SEQ ID NO:214, SEQ ID NO:216, SEQ ID NO:218, SEQ ID NO:220, SEQ ID NO:222, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:232, and SEQ ID NO:234 ("the polypeptide SEQ IDs"). While each of these polypeptide sequences are collectively referred to as "the polypeptide SEQ IDs" and are presented together in a group, each of these sequences can be separately considered and claimed.

Another embodiment of the invention provides a purified polypeptide comprising an amino acid sequence selected from the group consisting of "the polypeptide SEQ IDs."

Yet another embodiment of the invention provides a purified polynucleotide comprising a sequence that encodes a "polypeptide SEQ ID."

Still another embodiment of the invention provides a purified polynucleotide comprising at least about 15 contiguous nucleic acids of a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:159, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID

NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229,SEQ ID NO:231 and SEQ ID NO:233 ("the polynucleotide SEQ IDs"). While each of these polynucleotide sequences are collectively referred to as "the polynucleotide SEQ IDs" and are presented together in a group, each of these sequences can be separately considered and claimed.

Even another embodiment of the invention provides a purified polynucleotide comprising the nucleotide sequence of "the polynucleotide SEQ IDs" or degenerate variants thereof.

Another embodiment of the invention provides an expression vector comprising a "polynucleotide SEQ ID" operably linked to an expression control sequence. The vector can be in a cultured cell.

Still another embodiment of the invention provides an antibody or a fragment thereof that specifically binds to a polypeptide of "the polypeptide SEQ IDs." An antibody fragment can be, for example, a Fab or $F(ab')_2$ fragment. The antibody can be a monoclonal antibody or a polyclonal antibody. The antibody can be present in a pharmaceutical composition along with a pharmaceutically acceptable carrier.

Yet another embodiment of the invention provides a method for treating, ameliorating, or preventing a disease caused by *A. actinomycetemcomitans.* The method comprises administering to an animal an antibody of the invention or fragment thereof. A disease caused by *A.*

actinomycetemcomitans is thereby treated, ameliorated, or prevented. A disease caused by *A. actinomycetemcomitans* can be selected from the group consisting of localized prepubertal periodontis, generalized prepubertal periodontis, localized juvenile periodontis, generalized juvenile periodontis, rapidly progressive adult periodontis, refractory adult periodontis, endiocarditis, thyroid gland abscess, urinary tract infection, brain abscess and vertebral osteomyelitis.

Even another embodiment of the invention provides a method of detecting the presence of *A. actinomycetemcomitans* or an *A. actinomycetemcomitans* antigen in a test sample. The method comprises contacting a test sample with an antibody of the invention that specifically binds *A. actinomycetemcomitans* or an *A. actinomycetemcomitans* antigen under conditions that allow formation of an immunocomplex between the antibody and the *A. actinomycetemcomitans* or the *A. actinomycetemcomitans* antigen and detecting an immunocomplex. Detection of the immunocomplex indicates the presence of *A. actinomycetemcomitans* or an *A. actinomycetemcomitans* antigen in the test sample. The detected *A. actinomycetemcomitans* antigen can be an antigen that is expressed *in vivo* during infection of an animal.

Another embodiment of the invention provides a pharmaceutical composition that comprises a polypeptide of the invention and a pharmaceutically acceptable carrier.

Still another embodiment of the invention provides a method of eliciting an immune response. The method comprises administering a polypeptide of the invention to an animal, wherein an immune response is elicited.

Yet another embodiment of the invention provides a method of treating, preventing, or ameliorating a disease or infection caused by *A. actinomycetemcomitans*. The method comprises

administering a polypeptide of the invention to an animal, wherein the disease or infection is treated, prevented, or ameliorated.

Even another embodiment of the invention provides a composition comprising a polynucleotide of the invention and a pharmaceutically acceptable carrier. The polynucleotide can be DNA. The polynucleotide can be in a plasmid.

Another method of the invention provides a method of eliciting an immune response comprising administering a purified polynucleotide of the invention to an animal, wherein an immune response is elicited. Still another embodiment of the invention provides a method of treating, preventing, or ameliorating a disease or infection caused by *A. actinomycetemcomitans.* The method comprises administering a purified polynucleotide of the invention to an animal, wherein the disease or infection is treated, prevented, or ameliorated.

Yet another embodiment of the invention provides a method for identifying the presence of a first *A. actinomycetemcomitans* polynucleotide. The method comprises contacting a test sample suspected of containing a first *A. actinomycetemcomitans* polynucleotide with a second polynucleotide, wherein the second polynucleotide is a polynucleotide of the invention, under hybridization conditions. A hybridized first and second polynucleotide complex is detected. The presence of a hybridized first and second polynucleotide indicates the presence of a first polynucleotide in the test sample.

DETAILED DESCRIPTION OF THE INVENTION

Method of Identification of Polynucleotides and Polypeptides

A method for identifying nucleotide sequences that are important to a microorganism's ability to cause disease has been applied to Aa, the principal etiologic agent of early-onset

periodontitis including localized prepubertal periodontis, generalized prepubertal periodontis, localized juvenile periodontis, generalized juvenile periodontis, rapidly progressive adult periodontis, and refractory adult periodontis. Aa can also cause endocarditis, thyroid gland abscess, urinary tract infection, brain abscess and vertebral osteomyelitis. The method used to identify polynucleotide and polypeptide sequences of the invention is termed *in vivo* induced antigen technology (IVIAT). See Handfield *et al.*, Trends Microbiol. 336:336-339 (2000); WO 01/11081.

Briefly, IVIAT comprises obtaining a sample of antibodies against Aa antigens that are expressed by Aa *in vivo* and *in vitro* and adsorbing the antibodies with cells or cellular extracts of Aa that have been grown *in vitro*. An example of a sample of antibodies that can be used is sera from patients who have been or are infected with Aa. The unadsorbed antibodies are isolated and are used to probe an expression library of Aa DNA. Reactive clones are isolated and the cloned fragments sequenced.

IVIAT was used to identify polynucleotides of Aa that are expressed only when Aa is engaged in actually causing disease in animals, and in particular humans. Important environmental signals that normally cause Aa to turn on virulence genes during an infection are missing when the bacteria are grown in the laboratory. Therefore, many of the best targets for diagnostic and vaccine strategies were unknown. IVIAT methodology was used to identify polynucleotides that are specifically turned on during growth of Aa in a human host and not during routine laboratory growth. These polynucleotides and corresponding polypeptides and antibodies are useful in developing diagnostic tests for Aa to identify, for example, subjects who are in early stages of infection and for monitoring response to therapy, and for developing

vaccines or treatments to prevent or treat diseases caused by Aa in susceptible animals.

Aa antigens identified by IVIAT have a high predictive value with regard to diseases caused by Aa, for example, periodontal diseases. Diagnostic tests for Aa can be useful in applications such as screening children whose mothers have a history of periodontis to determine if the children have acquired a predisposition for the disease. Diseases known to be associated with periodontitis before puberty include Papillon-Lefevre syndrome (PLS), hypophosphatasia, neutropenias, leukocyte adhesion deficiency (LAD), Chediak-Higashi syndrome, Down's syndrome, leukemia, histiocytosis X, early-onset Type I diabetes, and acrodynia. Children with these diseases are candidates for Aa testing. Additionally, other preadolescent children who are less prone to periodontis would benefit from an Aa diagnostic test since there are no other predictors or known risk factors.

Polypeptides

Purified polypeptides of the invention can either be full-length polypeptides or fragments of polypeptides. For example, fragments of polypeptides of the invention can comprise about 5, 10, 25, 50, 100, or 200 amino acids of polypeptides of the invention. Examples of polypeptides of the invention include those shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID

NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:140, SEQ ID NO:142, SEQ ID NO:144, SEQ ID NO:146, SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:152, SEQ ID NO:154, SEQ ID NO:156, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:162, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:170, SEQ ID NO:172, SEQ ID NO:174, SEQ ID NO:176, SEQ ID NO:178, SEQ ID NO:180, SEQ ID NO:182, SEQ ID NO:184, SEQ ID NO:186, SEQ ID NO:188, SEQ ID NO:190, SEQ ID NO:192, SEQ ID NO:194, SEQ ID NO:196, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:208, SEQ ID NO:210, SEQ ID NO:212, SEQ ID NO:214, SEQ ID NO:216, SEQ ID NO:218, SEQ ID NO:220, SEQ ID NO:222, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:232, and SEQ ID NO:234. These polypeptides will be referred to as "the polypeptide SEQ IDs." Homologous amino acid sequences that are at least about 75, or about 90, 96, 98, or 99% identical to the polypeptide sequences shown in the polypeptide SEQ IDs are also Aa polypeptides. Homologous amino acid sequences retain biological activity, *i.e.,* are biologically functional equivalents.

Percent sequence identity has an art recognized meaning and there are a number of methods to measure identity between two polypeptide or polynucleotide sequences. *See, e.g.,*

Lesk, Ed., *Computational Molecular Biology*, Oxford University Press, New York, (1988); Smith, Ed., *Biocomputing: Informatics And Genome Projects*, Academic Press, New York, (1993); Griffin & Griffin, Eds., *Computer Analysis Of Sequence Data, Part I*, Humana Press, New Jersey, (1994); von Heinje, *Sequence Analysis In Molecular Biology*, Academic Press, (1987); and Gribskov & Devereux, Eds., *Sequence Analysis Primer*, M Stockton Press, New York, (1991). Methods for aligning polynucleotides or polypeptides are codified in computer programs, including the GCG program package (Devereux *et al.*, *Nuc. Acids Res.* 12:387 (1984)), BLASTP, BLASTN, FASTA (Atschul *et al.*, *J. Molec. Biol.* 215:403 (1990)), and Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) which uses the local homology algorithm of Smith and Waterman (*Adv. App. Math.*, 2:482-489 (1981)). For example, the computer program ALIGN which employs the FASTA algorithm can be used, with an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2.

When using any of the sequence alignment programs to determine whether a particular sequence is, for instance, about 95% identical to a reference sequence, the parameters are set such that the percentage of identity is calculated over the full length of the reference polynucleotide and that gaps in identity of up to 5% of the total number of nucleotides in the reference polynucleotide are allowed.

Variants of polypeptides shown in the polypeptide SEQ IDs and fragments thereof are also included in the invention. A variant is a polypeptide that differs from a polypeptide SEQ ID or fragment thereof, only in conservative substitutions and/or modifications, such that the antigenic properties of the polypeptide are substantially the same as the original polypeptide.

Variants can generally be identified by modifying one of the polypeptide sequences of the invention, and evaluating the antigenic properties of the modified polypeptide using, for example, an immunohistochemical assay, an enzyme-linked immunosorbant assay (ELISA), a radioimmunoassay (RIA), or a western blot assay. Polypeptides of the invention can comprise at least 1, 5, 10, 25, 50, or 100 conservative amino acid substitutions.

A conservative substitution is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

Variants can also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide can be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide can also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide can be conjugated to an immunoglobulin Fc region.

Polypeptides of the invention further comprise biologically functional equivalents of at least about 5, 10, 25, 50, 100, or 200 amino acids of the polypeptides shown in the polypeptide SEQ IDs. A polypeptide is a biological equivalent if it reacts substantially the same as a

polypeptide of the invention in an assay such as an immunohistochemical assay, an ELISA, an RIA, or a western blot assay, e.g. has 90-110% of the activity of the original polypeptide. In one embodiment, the assay is a competition assay wherein the biologically equivalent polypeptide is capable of reducing binding of the polypeptide of the invention to a corresponding reactive antigen or antibody by about 80, 95, 99, or 100%.

Polypeptides of the invention can comprise an antigen that is recognized by an antibody reactive against Aa. The antigen can comprise one or more epitopes (or antigenic determinants). An epitope can be a linear epitope, sequential epitope or a conformational epitope. Epitopes within a polypeptide of the invention can be identified by several methods. *See, e.g.,* U.S. Patent No. 4,554,101; Jameson & Wolf, *CABIOS* 4:181-186 (1988). For example, a polypeptide of the invention can be isolated and screened. A series of short peptides, which together span an entire polypeptide sequence, can be prepared by proteolytic cleavage. By starting with, for example, 100-mer polypeptide fragments, each fragment can be tested for the presence of epitopes recognized in an ELISA. For example, in an ELISA assay an Aa polypeptide, such as a 100-mer polypeptide fragment, is attached to a solid support, such as the wells of a plastic multi-well plate. A population of antibodies are labeled, added to the solid support and allowed to bind to the unlabeled antigen, under conditions where non-specific adsorbtion is blocked, and any unbound antibody and other proteins are washed away. Antibody binding is detected by, for example, a reaction that converts a colorless substrate into a colored reaction product. Progressively smaller and overlapping fragments can then be tested from an identified 100-mer to map the epitope of interest.

A polypeptide of the invention can be produced recombinantly. A polynucleotide encoding a polypeptide of the invention can be introduced into a recombinant expression vector, which can be expressed in a suitable expression host cell system using techniques well known in the art. A variety of bacterial, yeast, plant, mammalian, and insect expression systems are available in the art and any such expression system can be used. Optionally, a polynucleotide encoding a polypeptide can be translated in a cell-free translation system. A polypeptide can also be chemically synthesized.

If desired, a polypeptide can be produced as a fusion protein, which can also contain other amino acid sequences, such as amino acid linkers or signal sequences, as well as ligands useful in protein purification, such as glutathione-S-transferase, histidine tag, and staphylococcal protein A. More than one polypeptide of the invention can be present in a fusion protein.

Polynucleotides

Polynucleotides of the invention contain less than an entire microbial genome and can be single- or double-stranded DNA or RNA. The polynucleotides can be purified free of other components, such as proteins and lipids. The polynucleotides of the invention encode the polypeptides described above. Polynucleotides of the invention can also comprise other nucleotide sequences, such as sequences coding for linkers, signal sequences, heterologous signal sequences, TMR stop transfer sequences, transmembrane domains, or ligands useful in protein purification such as glutathione-S-transferase, histidine tag, and staphylococcal protein A.

Polynucleotides of the invention are shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ

ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:159, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, and SEQ ID NO:233. These polynucleotides will be referred to as the "polynucleotide SEQ IDs."

Degenerate nucleotide sequences encoding polypeptides of the invention, as well as homologous nucleotide sequences that are at least about 75, or about 90, 96, 98, or 99% identical to the nucleotide sequences shown in the polynucleotide SEQ IDs and the complements thereof are also polynucleotides of the invention. Percent sequence identity can be calculated as described in the "Polypeptides" section. Degenerate nucleotide sequences are polynucleotides that encode a polypeptide shown in the polypeptide SEQ IDs or fragments thereof, but differ in nucleic acid sequence from the sequence given in the polynucleotide SEQ IDs, due to the degeneracy of the genetic code. Complementary DNA (cDNA) molecules, species homologs, and variants of Aa polynucleotides that encode biologically functional Aa polypeptides also are Aa polynucleotides. A polynucleotide of the invention can comprise about 5, 10, 15, 50, 100, or 200 nucleotides of a nucleic acid sequence shown in the polynucleotide SEQ IDs.

Polynucleotides of the invention can be isolated from nucleic acid sequences present in, for example, a biological sample, such as plaque, saliva, crevicular fluid, sputum, blood, serum, plasma, urine, feces, cerebrospinal fluid, amniotic fluid, wound exudate, or tissue, from an infected individual. Polynucleotides can also be synthesized in the laboratory, for example, using an automatic synthesizer. An amplification method such as PCR can be used to amplify polynucleotides from either genomic DNA or cDNA encoding the polypeptides.

Polynucleotides of the invention can comprise coding sequences for naturally occurring polypeptides or can encode altered sequences which do not occur in nature. If desired, polynucleotides can be cloned into an expression vector comprising expression control elements, including for example, origins of replication, promoters, enhancers, or other regulatory elements that drive expression of the polynucleotides of the invention in host cells. An expression vector

can be, for example, a plasmid, such as pBR322, pUC, or ColE1, or an adenovirus vector, such as an adenovirus Type 2 vector or Type 5 vector. Optionally, other vectors can be used, including but not limited to Sindbis virus, simian virus 40, alphavirus vectors, poxvirus vectors, and cytomegalovirus and retroviral vectors, such as murine sarcoma virus, mouse mammary tumor virus, Moloney murine leukemia virus, and Rous sarcoma virus. Minichromosomes such as MC and MC1, bacteriophages, phagemids, yeast artificial chromosomes, bacterial artificial chromosomes, virus particles, virus-like particles, cosmids (plasmids into which phage lambda *cos* sites have been inserted) and replicons (genetic elements that are capable of replication under their own control in a cell) can also be used.

Methods for preparing polynucleotides operably linked to an expression control sequence and expressing them in a host cell are well-known in the art. *See, e.g.,* U.S. Patent No. 4,366,246. A polynucleotide of the invention is operably linked when it is positioned adjacent to one or more expression control elements, which direct transcription and/or translation of the polynucleotide.

A vector comprising a polynucleotide of the invention can be transformed into, for example, bacterial, yeast, insect, or mammalian cells so that the polypeptides of the invention can be expressed in and isolated from cell culture. Any of those techniques that are available in the art can be used to introduce polynucleotides into the host cells. These include, but are not limited to, transfection with naked or encapsulated nucleic acids, cellular fusion, protoplast fusion, viral infection, and electroporation.

Polynucleotides of the invention can be used, for example, as probes or primers, for example PCR primers, to detect the presence of Aa polynucleotides in a sample, such as a

biological sample. The ability of such probes and primers to specifically hybridize to Aa polynucleotide sequences will enable them to be of use in detecting the presence of complementary sequences in a given sample. Polynucleotide probes and primers of the invention can hybridize to complementary sequences in a sample such as a biological sample, including plaque, saliva, crevicular fluid, sputum, blood, urine, feces, cerebrospinal fluid, amniotic fluid, wound exudate, or tissue. Polynucleotides from the sample can be, for example, subjected to gel electrophoresis or other size separation techniques or can be immobilized without size separation. The polynucleotide probes or primers can be labeled. Suitable labels, and methods for labeling probes and primers are known in the art, and include, for example, radioactive labels incorporated by nick translation or by kinase, biotin labels, fluorescent labels, chemiluminescent labels, bioluminescent labels, metal chelator labels and enzyme labels. The polynucleotides from the sample are contacted with the probes or primers under hybridization conditions of suitable stringencies.

Depending on the application, varying conditions of hybridization can be used to achieve varying degrees of selectivity of the probe or primer towards the target sequence. For applications requiring high selectivity, relatively stringent conditions can be used, such as low salt and/or high temperature conditions, such as provided by a salt concentration of from about 0.02 M to about 0.15 M salt at temperatures of from about 50°C to about 70°C. For applications requiring less selectivity, less stringent hybridization conditions can be used. For example, salt conditions from about 0.14 M to about 0.9M salt, at temperatures ranging form about 20°C to about 55°C. The presence of a hybridized complex comprising the probe or primer and a

complementary polynucleotide from the test sample indicates the presence of Aa or an Aa polynucleotide sequence in the sample.

Antibodies

Antibodies of the invention are antibody molecules that specifically and stably bind to an Aa polypeptide of the invention or fragment thereof. An antibody of the invention can be a polyclonal antibody, a monoclonal antibody, a single chain antibody (scFv), or a fragment of an antibody. Fragments of antibodies are a portion of an intact antibody comprising the antigen binding site or variable region of an intact antibody, wherein the portion is free of the constant heavy chain domains of the Fc region of the intact antibody. Examples of antibody fragments include Fab, Fab', Fab'-SH, $F(ab')_2$ and F_v fragments.

An antibody of the invention can be any antibody class, including for example, IgG, IgM, IgA, IgD and IgE. An antibody or fragment thereof binds to an epitope of a polypeptide of the invention. An antibody can be made *in vivo* in suitable laboratory animals or *in vitro* using recombinant DNA techniques. Means for preparing and characterizing antibodies are well know in the art. *See, e.g.,* Dean, *Methods Mol. Biol.* 80:23-37 (1998); Dean, *Methods Mol. Biol.* 32:361-79 (1994); Baileg, *Methods Mol. Biol.* 32:381-88 (1994); Gullick, *Methods Mol. Biol.* 32:389-99 (1994); Drenckhahn *et al. Methods Cell. Biol.* 37:7-56 (1993); Morrison, *Ann. Rev. Immunol.* 10:239-65 (1992); Wright *et al. Crit. Rev. Immunol.* 12:125-68(1992). For example, polyclonal antibodies can be produced by administering a polypeptide of the invention to an animal, such as a human or other primate, mouse, rat, rabbit, guinea pig, goat, pig, cow, sheep, donkey, or horse. Serum from the immunized animal is collected and the antibodies are purified from the plasma by, for example, precipitation with ammonium sulfate, followed by

chromatography, such as affinity chromatography. Techniques for producing and processing polyclonal antibodies are known in the art.

Additionally, monoclonal antibodies directed against epitopes present on a polypeptide of the invention can also be readily produced. For example, normal B cells from a mammal, such as a mouse, which was immunized with a polypeptide of the invention can be fused with, for example, HAT-sensitive mouse myeloma cells to produce hybridomas. Hybridomas producing Aa-specific antibodies can be identified using RIA or ELISA and isolated by cloning in semi-solid agar or by limiting dilution. Clones producing Aa-specific antibodies are isolated by another round of screening. Monoclonal antibodies can be screened for specificity using standard techniques, for example, by binding a polypeptide of the invention to a microtiter plate and measuring binding of the monoclonal antibody by an ELISA assay. Techniques for producing and processing monoclonal antibodies are known in the art. See e.g., Kohler & Milstein, Nature, 256:495 (1975). Particular isotypes of a monoclonal antibody can be prepared directly, by selecting from the initial fusion, or prepared secondarily, from a parental hybridoma secreting a monoclonal antibody of a different isotype by using a sib selection technique to isolate class-switch variants. *See* Steplewski *et al., P.N.A.S. U.S.A.* 82:8653 1985; Spria *et al., J. Immunolog. Meth.* 74:307, 1984. Monoclonal antibodies of the invention can also be recombinant monoclonal antibodies. *See, e.g.,* U.S. Patent No. 4,474,893; U.S. Patent No. 4,816,567. Antibodies of the invention can also be chemically constructed. *See, e.g.,* U.S. Patent No. 4,676,980.

Antibodies of the invention can be chimeric (*see, e.g.,* U.S. Patent No. 5,482,856), humanized (*see, e.g.,* Jones *et al., Nature* 321:522 (1986); Reichmann *et al., Nature* 332:323

(1988); Presta, *Curr. Op. Struct. Biol.* 2:593 (1992)), or human antibodies. Human antibodies can be made by, for example, direct immortilization, phage display, transgenic mice, or a Trimera methodology, *see e.g.,* Reisener *et al., Trends Biotechnol.* 16:242-246 (1998).

Antibodies, either monoclonal and polyclonal, which are directed against Aa antigens, are particularly useful for detecting the presence of Aa or Aa antigens in a sample, such as a serum sample from an Aa-infected human. An immunoassay for Aa or an Aa antigen can utilize one antibody or several antibodies. An immunoassay for Aa or an Aa antigen can use, for example, a monoclonal antibody directed towards an Aa epitope, a combination of monoclonal antibodies directed towards epitopes of one Aa polypeptide, monoclonal antibodies directed towards epitopes of different Aa polypeptides, polyclonal antibodies directed towards the same Aa antigen, polyclonal antibodies directed towards different Aa antigens, or a combination of monoclonal and polyclonal antibodies. Immunoassay protocols can be based upon, for example, competition, direct reaction, or sandwich type assays using, for example, labeled antibody. Antibodies of the invention can be labeled with any type of label known in the art, including, for example, fluorescent, chemiluminescent, radioactive, enzyme, colloidal metal, radioisotope and bioluminescent labels.

Antibodies of the invention or fragments thereof can be bound to a support and used to detect the presence of Aa or an Aa antigen. Supports include, for example, glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magletite.

Polyclonal or monoclonal antibodies of the invention can further be used to isolate Aa organisms or Aa antigens by immunoaffinity columns. The antibodies can be affixed to a solid

support by, for example, adsorbtion or by covalent linkage so that the antibodies retain their immunoselective activity. Optionally, spacer groups can be included so that the antigen binding site of the antibody remains accessible. The immobilized antibodies can then be used to bind Aa organisms or Aa antigens from a sample, such as a biological sample including saliva, plaque, crevicular fluid, sputum, blood, urine, feces, cerebrospinal fluid, amniotic fluid, wound exudate, or tissue. The bound Aa organisms or Aa antigens are recovered from the column matrix by, for example, a change in pH.

Antibodies of the invention can also be used in immunolocalization studies to analyze the presence and distribution of a polypeptide of the invention during various cellular events or physiological conditions. Antibodies can also be used to identify molecules involved in passive immunization and to identify molecules involved in the biosynthesis of non-protein antigens. Identification of such molecules can be useful in vaccine development. Antibodies of the invention, including, for example, monoclonal antibodies and single chain antibodies, can be used to monitor the course of amelioration of a disease caused by Aa. By measuring the increase or decrease of Aa antibodies to Aa proteins in a test sample from an animal, it can be determined whether a particular therapeutic regiment aimed at ameliorating the disorder is effective. Antibodies can be detected and/or quantified using for example, direct binding assays such as RIA, ELISA, or western blot assays.

An antibody of the invention can be used in a method of the diagnosis of Aa infection by obtaining a test sample from an animal suspected of having an Aa infection. The test sample is contacted with an antibody of the invention under conditions enabling the formation of an antibody-antigen complex (*i.e.*, an immunocomplex). The amount of antibody-antigen

complexes can be determined by methodology known in the art. A level that is higher than that formed in a control sample indicates an Aa infection. Alternatively, a polypeptide of the invention can be contacted with a test sample. Aa antibodies in a positive body sample will form an antigen-antibody complex under suitable conditions. The amount of antibody-antigen complexes can be determined by methods known in the art.

Methods of Treatment, Amelioration, or Prevention of a Disease Caused by Aa

Polypeptides, polynucleotides, and antibodies of the invention can be used to treat, ameliorate, or prevent a disease caused by Aa, such as early-onset periodontitis including localized and generalized prepubertal periodontis, localized and generalized juvenile periodontis, and rapidly progressive or refractory adult periodontitis, endocarditis, thyroid gland abscesses, urinary tract infections, brain abscesses, and vertebral osteomyelitis.

For example, an antibody, such as a monoclonal antibody of the invention or fragments thereof, can be administered to an animal, such as a human. In one embodiment of the invention an antibody or fragment thereof is administered to an animal in a pharmaceutical composition comprising a pharmaceutically acceptable carrier. A pharmaceutical composition comprises a therapeutically effective amount of an antibody or fragments thereof. A therapeutically effective amount is an amount effective in alleviating the symptoms of Aa infection or in reducing the amount of Aa organisms in a subject.

Polypeptides or polynucleotides of the invention can be used to elicit an immune response in a host. An immonogenic polypeptide or polynucleotide is a polypeptide or polynucleotide of the invention that is capable of inducing an immune response in an animal. An immunogenic polypeptide or polynucleotide of the invention is particularly useful in sensitizing an immune

system of an animal such that, as one result, an immune response is produced that ameliorates or prevents the effect of Aa infection. The elicitation of an immune response in animal model can be useful to determine, for example, optimal doses or administration routes. Elicitation of an immune response can also be used to treat, prevent, or ameliorate a disease or infection caused by Aa. An immune response includes humoral immune responses or cell mediated immune responses, or a combination thereof. An immune response can also comprise the promotion of a generalized host response, e.g., by promoting the production of defensins.

The generation of an antibody titer by an animal against Aa can be important in protection from infection and clearance of infection. Detection and/or quantification of antibody titers after delivery of a polypeptide or polynucleotide can be used to identify epitopes that are particularly effective at eliciting antibody titers. Epitopes responsible for a strong antibody response to Aa can be identified by eliciting antibodies directed against Aa polypeptides of different lengths. Antibodies elicited by a particular polypeptide epitope can then be tested using, for example, an ELISA assay to determine which polypeptides contain epitopes that are most effective at generating a strong response. Polypeptides or fusion proteins that contain these epitopes or polynucleotides encoding the epitopes can then be constructed and used to elicit a strong antibody response.

A polypeptide, polynucleotide, or antibody of the invention can be administered to a mammal, such as a mouse, rabbit, guinea pig, macaque, baboon, chimpanzee, human, cow, sheep, pig, horse, dog, cat, or to animals such as chickens or ducks, to elicit antibodies *in vivo*. Injection of a polynucleotide has the practical advantages of simplicity of construction and modification. Further, injection of a polynucleotide results in the synthesis of a polypeptide in

the host. Thus, the polypeptide is presented to the host immune system with native post-translational modifications, structure, and conformation. A polynucleotide can be delivered to a subject as "naked DNA."

Administration of a polynucleotide, polypeptide, or antibody can be by any means known in the art, including intramuscular, intravenous, intrapulmonary, intramuscular, intradermal, intraperitoneal, or subcutaneous injection, aerosol, intranasal, infusion pump, suppository, mucosal, topical, and oral, including injection using a biological ballistic gun ("gene gun"). A polynucleotide, polypeptide, or antibody can be accompanied by a protein carrier for oral administration. A combination of administration methods can also be used to elicit an immune response. Antibodies can be administered at a daily dose of about 0.5 mg to about 200 mg. In one embodiment of the invention antibodies are administered at a daily dose of about 20 to about 100 mg.

Pharmaceutically acceptable carriers and diluents for therapeutic use are well known in the art and are described in, for example, Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro ed. (1985)). The carrier should not itself induce the production of antibodies harmful to the host. Such carriers include, but are not limited to, large, slowly metabolized, macromolecules, such as proteins, polysaccharides such as latex functionalized sepharose, agarose, cellulose, cellulose beads and the like, polylactic acids, polyglycolic acids, polymeric amino acids such as polyglutamic acid, polylysine, and the like, amino acid copolymers, peptoids, lipitoids, and inactive, avirulent virus particles or bacterial cells. Liposomes, hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesives can also be used as a carrier for a composition of the invention.

Pharmaceutically acceptable salts can also be used in compositions of the invention, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as salts of organic acids such as acetates, proprionates, malonates, or benzoates. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those of skill in the art. Compositions of the invention can also contain liquids or excipients, such as water, saline, phosphate buffered saline, Ringer's solution, Hank's solution, glucose, glycerol, dextrose, malodextrin, ethanol, or the like, singly or in combination, as well as substances such as wetting agents, emulsifying agents, tonicity adjusting agents, detergent, or pH buffering agents. Additional active agents, such as bacteriocidal agents can also be used.

If desired, co-stimulatory molecules, which improve immunogen presentation to lymphocytes, such as B7-1 or B7-2, or cytokines such as MIP1α, GM-CSF, IL-2, and IL-12, can be included in a composition of the invention. Optionally, adjuvants can also be included in a composition. Adjuvants are substances that can be used to nonspecifically augment a specific immune response. Generally, an adjuvant and a polypeptide of the invention are mixed prior to presentation to the immune system, or presented separately, but are presented into the same site of the animal. Adjuvants can include, for example, oil adjuvants (*e.g.* Freund's complete and incomplete adjuvants) mineral salts (*e.g.* $Alk(SO_4)_2$; $AlNa(SO_4)_2$, $AlNH_4(SO_4)$, Silica, Alum, $Al(OH)_3$, and $Ca_3(PO_4)_2$), polynucleotides (*i.e.* Polyic and Poly AU acids), and certain natural substances (*e.g.* wax D from *Mycobacterium tuberculosis*, as well as substances found in *Corynebacterium parvum*, *Bordetella pertussis* and members of the genus Brucella. Adjuvants which can be used include, but are not limited to MF59-0, aluminum hydroxide, N-acetyl-

muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637), referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn –glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion.

The compositions of the invention can be formulated into ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, injectable formulations, mouthwashes, dentrifices, and the like. The percentage of one or more polypeptides, polynucleotides, or antibodies of the invention in such compositions and preparations can vary from 0.1% to 60% of the weight of the unit.

Administration of polypeptides or polynucleotides can elicit an immune response in the animal that lasts for at least 1 week, 1 month, 3 months, 6 months, 1 year, or longer. Optionally, an immune response can be maintained in an animal by providing one or more booster injections of the polypeptide or polynucleotide at 1 month, 3 months, 6 months, 1 year, or more after the primary injection. If desired, co-stimulatory molecules or adjuvants can also be provided before, after, or together with the compositions.

A composition of the invention comprising a polypeptide, polynucleotide, or a combination thereof is administered in a manner compatible with the particular composition used and in an amount that is effective to elicit an immune response as detected by, for example, an ELISA. A polynucleotide can be injected intramuscularly to a large mammal, such as a baboon, chimpanzee, or human, at a dose of 1 ng/kg, 10 ng/kg, 100 ng/kg, 1000 ng/kg, 0.001 mg/kg, 0.1

mg/kg, or 0.5 mg/kg. A polypeptide can be injected intramuscularly to a large mammal, such as a human, at a dose of 0.01, 0.05, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 5 or 10 mg/kg.

Polypeptides, polynucleotides, or antibodies, or a combination thereof can be administered either to an animal that is not infected with Aa or can be administered to an Aa-infected animal. The particular dosages of polynucleotide, polypeptides, or antibodies in a composition will depend on many factors including, but not limited to the species, age, gender, concurrent medication, general condition of the mammal to which the composition is administered, and the mode of administration of the composition. An effective amount of the composition of the invention can be readily determined using only routine experimentation.

The materials for use in a method of the invention can be present in a kit. A kit can comprise one or more elements used in the method. For example, a kit can contain an antibody of the invention in a container and Aa polypeptides in another container. The kit and containers are laled with their contents and the kit includes instructions for use of the elements in the containers. The constituents of the kit can be present in, for example, liquid or lypholized form.

All references cited in this disclosure are incorporated herein by reference.